A Tale of Two Lymphomas:
Case discussions emphasizing the latest in diagnosis, classification and
treatment of Canine Lymphoma
Amy Durham, MS, VMD, DACVP
amycd@vet.upenn.edu
Jennifer Mahoney, DVM, DACVIM (Oncology)
jamah@vet.upenn.edu
Nicola Mason, BVetMed, PhD, DACVIM (SAIM)
njmason@vet.upenn.edu
University of Pennsylvania, Philadelphia, PA, USA

Introduction

Hematopoietic neoplasia is a large and diverse group of clonal proliferative disorders of hematopoietic cells. The most common hematopoietic malignancy in dogs is lymphoma. While multicentric, large cell lymphoma represents the most common clinical presentation, lymphoma can affect a wide range of organ systems, causing a variety of clinical signs. The multicentric lymphoma that we see in our canine patients is most similar to Non-Hodgkin’s lymphoma in humans, a disease that is further categorized into several subtypes based on the World Health Organization (WHO) classification system (see below). Classification/subtyping of lymphoma can provide valuable prognostic information and help direct treatment.

Diagnostic Work-Up

For patients presenting with an enlarged lymph node or nodes, fine needle aspiration and submission for cytology is a reasonable diagnostic starting point. If lymphoma is confirmed or suspected based on cytology, other diagnostic tests are warranted. These tests are important for determining stage, lymphoma subtype, and prognosis, but are also important in determining a patient’s ability to tolerate treatment. It is important to note that complete lymphoma subtyping can only be performed on histopathology sections and accompanying immunophenotyping.

Staging

A complete minimum database includes a complete blood count (CBC), chemistry panel, and urinalysis. The CBC can help detect any evidence of bone marrow involvement, which may indicate a need for bone marrow evaluation through an aspirate and possibly a core biopsy. The chemistry panel is important for detecting the presence of hypercalcemia, which raises suspicion for T cell lymphoma. Liver enzymes and liver function indicators should be evaluated carefully, as most chemotherapy drugs are metabolized by the liver. In addition, azotemic patients may be at increased risk for developing acute tumor lysis syndrome after initiation of treatment, which may change the plan for chemotherapy induction, or suggest a need for concurrent fluid diuresis.
Imaging with thoracic radiographs and abdominal ultrasound is also recommended as part of our complete staging of a lymphoma patient. Thoracic radiographs are evaluated for the presence of a cranial mediastinal mass (common in T cell lymphoma), enlarged tracheobronchial or sternal lymph nodes, or pulmonary infiltration of lymphoma. Abdominal ultrasound is used to evaluate the appearance of the liver and spleen, as well as to identify any enlarged intra-abdominal lymph nodes. Organs such as the kidneys, gastrointestinal tract, and pancreas may also be affected with lymphoma. Aspirates of the liver and spleen may be performed as indicated to confirm the presence of lymphoma in these organs.

After completion of the diagnostics above, we now have enough information to stage our lymphoma patient. The WHO staging system for canine lymphoma is as follows:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Sites Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Single lymph node or lymphoid organ involvement</td>
</tr>
<tr>
<td>II</td>
<td>Regional lymph node involvement, on the same side of the diaphragm</td>
</tr>
<tr>
<td>III</td>
<td>Generalized lymph node involvement (both sides of the diaphragm)</td>
</tr>
<tr>
<td>IV</td>
<td>Liver and/or Spleen involvement, +/- generalized disease</td>
</tr>
<tr>
<td>V</td>
<td>Bone marrow or any other site (pulmonary, CNS, renal, etc), +/- generalized disease</td>
</tr>
</tbody>
</table>

In addition to Stage, patients are also classified into one of two Substages: Substage “a,” a patient who is clinically feeling well, or Substage “b,” a patient who is clinically ill.

Pathology

Historically, numerous systems have been used to classify hematopoietic neoplasms in human medicine, some of which have been applied inconsistently to veterinary species (examples include the Kiel classification and National Cancer Institute Working Formulation). The World Health Organization (WHO) classification of hematopoietic neoplasia was first published in 2001 (updated in 2008) and is based on the principles defined in the Revised European-American Classification of Lymphoid Neoplasms (REAL). This classification system is considered the first true worldwide consensus on the classification of hematopoietic malignancies and currently accepted as the method of choice in both human and veterinary medicine.

The WHO classification integrates information on tumor topography, cell morphology, immunophenotype, genetic features, and clinical presentation and course. It broadly categorizes neoplasms primarily according to cell lineage: myeloid, lymphoid, and histiocytic. Leukemias are often further distinguished and refer to a group of hematopoietic neoplasms that arise from the bone marrow and are present within the blood. Therefore, leukemia may be difficult to differentiate from other forms of hematopoietic neoplasms that originate outside of the bone marrow but infiltrate the bone marrow and blood. Those cases of secondary bone marrow or blood involvement may not be considered leukemia but rather the “leukemic phase” of another primary
neoplasm. The WHO system recognizes that certain lymphomas and leukemias are different manifestations of the same disease (e.g., chronic lymphocytic leukemia and small lymphocytic lymphoma), and the designation of lymphoma or leukemia is placed on the tissue with the largest tumor burden.

Lymphomas are a diverse group of malignancies arising in lymphoid tissue outside of bone marrow. Because of the great variation in the clinical manifestations, behavior, and pathologic features of lymphoma, classification is crucial to better direct therapy and to predict clinical response and outcome. The WHO classification of lymphoma postulates a normal cell counterpart for each type of lymphoma, which acknowledges that lymphoma can arise at any stage in the development/maturation of a lymphocyte. In applying the WHO system, pathologists use gross and histomorphologic features, immunophenotype (B or T lymphocyte), and clinical characteristics to classify lymphomas. The features used in histopathologic classification of biopsy samples are tumor architecture (nodular or diffuse); cell size (small-intermediate-large) based on comparison with a red blood cell (*note, cytopathologists use the size of a neutrophil for measurement); and grade based on the number of mitotic figures in a single high-power field (indolent-low-mid-high).

Although there are numerous subtypes of lymphoma recognized under the WHO system, a detailed discussion of each subtype is outside the scope of this paper. However, a select number of subtypes are more commonly seen and several lymphoma subtypes are identified in dogs and clinically range from slow-growing indolent tumors to highly aggressive tumors. The most common types in dogs are large cell lymphomas and include diffuse large B cell lymphoma and peripheral T cell lymphoma. Intermediate cell lymphomas include B or T cell lymphoblastic lymphomas and Burkitt-like lymphoma (both high grade), marginal zone lymphoma, and the intermediate cell variant of T zone lymphomas (both indolent and nodular). The most common small cell lymphomas in dogs are T zone lymphoma (small cell variant), and small lymphocytic lymphoma. Cutaneous lymphomas are most often of T lymphocyte origin and may be epitheliotropic or nonepitheliotropic, and a distinct entity of inflamed T lymphocyte lymphoma has been recently described in dogs.

Diffuse Large B Cell Lymphoma (DLBCL) comprise up to half of all cases in dogs, which are mid- to high-grade lymphomas. Histologically, lymph node architecture is most often completely effaced by sheets of large neoplastic cells, which may invade through the capsule and colonize the perinodal tissue. Peripheral T cell lymphomas (PTCL) are the second most common subtype in dogs. This category includes all T cell lymphomas that do not fit into the other categories (e.g., T zone lymphoma, enteropathy-associated T cell lymphoma, and hepatosplenic T cell lymphoma). Peripheral T cell lymphoma also effaces nodal architecture, and when compared to diffuse large B cell lymphoma, there is more variation in nuclear size and morphologic features. Lymphoblastic lymphoma (LBL – B or T cell) and Burkitt-like lymphoma (BLL – B cell) are both intermediate cell size, and are less common high-grade lymphomas compared to DLBCL and PTCL in dogs. T cell LBL is more common than B cell LBL, and is considered an aggressive, treatment-resistant disease.
Indolent lymphomas constitute up to 29% of all canine lymphomas and include (in descending order of frequency) T zone lymphoma (TZL), marginal zone lymphoma (MZL), mantle cell lymphoma (MCL), and follicular lymphoma (FL). T zone lymphoma is the most common indolent lymphoma in dogs and can present as a solitary or multiple enlarge lymph node(s) (often mandibular lymph nodes) in healthy-appearing dogs. The characteristic histopathologic architecture is a nodular expansion of the paracortex by small to intermediate sized neoplastic cells. Mitotic figures are rare, which is why it has an indolent grade. Dogs with TZL may be diagnosed with an advanced stage of disease yet remain clinically healthy, without loss of appetite or activity level. Dogs with T zone lymphoma have a relatively long survival time compared to other lymphomas.

Specific Pathology Tests:
Immunophenotype can be determined via flow cytometry, immunocytochemistry (ICC) of cytologic slides, or immunohistochemistry (IHC) of biopsy samples (most often formalin fixed paraffin embedded tissues). The most commonly used IHC markers are CD79a, CD20 and Pax5 for B cells and CD3 for T cells. Flow cytometry can be performed from any suspension of cells in fluid, for example a lymph node aspiration suspended in special flow cytometry media, or blood from a suspected leukemic patient. Samples must be shipped overnight to the diagnostic lab, as cells are only viable for about 48 hours. Flow cytometry provides detailed information on the percentage of lymphocytes that express specific cluster of differentiation (CD) markers. In addition to the B and T cell markers listed above, flow cytometry often evaluates for additional markers such as CD34 and CD45, which can provide valuable prognostic information. CD34 is a marker of precursor cells of both lymphoid and myeloid lineages and helps to distinguish acute leukemia from Stage V lymphoma. Loss of CD45 has been associated with T cell lymphomas that exhibit a more indolent clinical course (e.g. T zone lymphoma).

Polymerase chain reaction (PCR) for antigen receptor rearrangement (PARR) is a technique that amplifies DNA from the variable regions of the immunoglobulin or T-cell receptor genes. PARR is recommended in cases of suspected lymphoma and in conjunction with a separate immunophenotyping test (e.g. flow, ICC, IHC); PARR results should never be interpreted in isolation. This DNA can be extracted from a cytology preparation slide or biopsy sample. Clonality is the hallmark of cancer, so dogs with B cell lymphoma should have identical DNA sequences of their immunoglobulin receptor gene, while dogs with T cell lymphoma should have identical DNA sequences of their T-cell receptor gene. A single band on a PCR gel indicates a clonal lymphocyte population and helps to confirm a diagnosis of lymphoma.
Treatment and Prognosis

*Multicentric lymphoma*

In most cases, canine lymphoma presents as a multicentric disease, and systemic chemotherapy is therefore still the mainstay of treatment. Combination chemotherapy protocols have been found to be more effective than single-agent protocols, most likely because treating with multiple agents with different mechanisms of action delays tumor cell resistance. CHOP-based chemotherapy protocols (cyclophosphamide, doxorubicin, vincristine, prednisone) are the standard of care, with remission rates of 60-90% and median remission times of 6-12 months depending on the tumor phenotype and the particular protocol used. Dogs with PTCL tend to develop resistance to chemotherapy much earlier than dogs with DLBCL, resulting in shorter remission and survival times. A small number of studies specifically evaluating outcome for dogs with PTCL treated with CHOP-based protocols report a median progression-free survival of 3-7 months, and overall survival time of 4-8 months. This is in contrast to a median survival time of 10-12 months for dogs with DLBCL treated with CHOP-based chemotherapy.

Most patients tolerate chemotherapy well, although dogs can experience gastrointestinal upset and bone marrow suppression. Certain breeds with continuous (anagen-phase predominant) hair growth may also be prone to hair loss. Each of the drugs in the CHOP protocol has its own unique potential side effects, such as cardiotoxicity from doxorubicin or sterile hemorrhagic cystitis from cyclophosphamide. The risk of side effects from a particular drug must be considered for each individual patient when administering a chemotherapy protocol, and doses must be adjusted as necessary if a patient experiences severe side effects. Prophylactic and/or supportive care with antinausea medications, antidiarrheal medications, and antibiotics are provided as needed throughout the treatment protocol to help manage side effects.

Some owners have concerns about the finances of a CHOP-based protocol, or about the scheduling and frequency of visits for chemotherapy. Alternatives to CHOP-based protocols include single-agent doxorubicin (remission rate 60-85%, median remission time 4-7 months); lomustine/prednisone (remission rate 52%, median remission time 3.5 months); COP (cyclophosphamide, vincristine, prednisone; remission rate 70-75%, median remission time 3-6 months); or prednisone alone (median remission 1-2 months).

L-asparaginase was previously incorporated into many CHOP-based protocols, but recently has become available only in compounded form. While the compounded form seems to be effective, the shortage of L-asparaginase prompted many oncologists to reserve this treatment for patients who were clinically ill at diagnosis, or for patients who have come out of remission. L-asparaginase resistance develops by formation of antibodies, usually after 2-3 treatments. Many oncologists now feel there is an advantage to “saving” L-asparaginase for later in the course of treatment, and are omitting its use from the induction of patients who feel clinically well at the time of diagnosis.
It is also important to carefully educate clients about the use of prednisone. Prednisone should not be started until a definitive diagnosis of lymphoma has been obtained. Prednisone can mask the signs of lymphoma, and even if a cytologic diagnosis has been made, steroids may impact results of more advanced diagnostics such as imaging, immunophenotyping, or histopathology. Prednisone can also induce multiple drug resistance (MDR), decreasing response to chemotherapy drugs such as L-asparaginase, vincristine, and doxorubicin.

**Indolent lymphoma**

Many forms of indolent lymphoma do not require systemic chemotherapy and can be approached with watchful waiting. Median survival times for dogs with CD45 negative T zone lymphoma have been reported as 13-33 months, while median survival time for dogs with splenic marginal zone lymphoma is reported as 38 months with splenectomy alone. Because these lymphoma cells divide more slowly, they are less susceptible to maximal-tolerated dose chemotherapy. If chemotherapy is considered, such as in cases with multicentric disease or concurrent leukemia, chlorambucil and prednisone protocols are preferable to CHOP-based protocols. However, one study found that the use of systemic treatment did not influence survival. Further clinical studies are needed to better determine the effect of chemotherapy on indolent lymphomas. The significant difference in behavior between indolent lymphomas and aggressive, high grade lymphomas demonstrates the important role that full histologic classification can play in the treatment of these cases. If a patient presents with a more chronic disease course, or if cytology results are inconclusive, a lymph node biopsy can provide valuable information in directing treatment of these patients.

**Selected References:**


